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Mesabolivar brasiliensis (Moenkhaus 1898) and *Mesabolivar cyaneotaeniatus* (Keyserling 1891) (Araneomorphae, Pholcidae): close relationship reinforced by cytogenetic analyses

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Abstract. Pholcidae is the most diverse family among haplogyne spiders but only 15 species have been analyzed cytogenetically. These studies revealed that the diploid number varies from 2n = 15 to 2n = 32, that there are three types of sex chromosome systems in males (X, X_1X_2 and X_1X_2Y), and that the chromosomes are predominantly biarmed. Within the genus *Mesabolivar*, only *Mesabolivar luteus* (Keyserling 1891) has been karyotyped, and it showed 2n = 15 = 14 + X, with all chromosomes being metacentric. In the present work, we characterize the mitotic and meiotic chromosomes of *Mesabolivar brasiliensis* (Moenkhaus 1898) and *Mesabolivar cyaneotaeniatus* (Keyserling 1891). Male mitotic metaphases of the two species showed the diploid number 2n = 17 = 16 + X; oogonial cells of *M. brasiliensis* showed 2n = 18 = 16 + XX. In both species, the chromosomes were exclusively biarmed, and the X chromosome was the largest element of the karyotype. Diplotene spermatocytes of the two species exhibited 8II + X and the occurrence of only one terminal or interstitial chiasma per bivalent. In *M. cyaneotaeniaus*, metaphases II with n = 9 = 8 + X and n = 8 were found, indicating the regular segregation of all chromosomes during meiosis I. Mitotic metaphases of *M. brasiliensis* stained with CMA_3/DA/DAPI revealed GC-rich chromatin in the terminal region of almost all autosomes, especially in pair 2. An earlier revision of the New World pholcids grouped *M. brasiliensis* and *M. cyaneotaeniatus* in a "southern group" and placed *M. luteus* in a "miscellaneous group." A molecular study showed a closer relationship between *M. brasiliensis* and *M. cyaneotaeniatus* than between *M. luteus* and either of these two species. The 2n = 17 found in *M. brasiliensis* and *M. cyaneotaeniatus* corroborates this hypothesis, given that *M. luteus* has a diploid number of 2n = 15.

Keywords: Chromosome, Haplogynae, meiosis, sex chromosome system, spider

According to Platnick (2008), the family Pholcidae Koch 1851 includes 999 extant species, and thus constitutes the most diverse family among haplogyne spiders. A phylogenetic analysis based on morphological characters separated pholcids into four clades: "ninetines," "pholcines," "holocnemines," and the "New World clade" (Huber 2000). The genus *Mesabolivar* González-Sponga 1998, which ranges from northern South America to northern Argentina, is included in the "New World clade" and possesses 45 species (Huber 2000, 2008). Moreover, the genus is divided into four operational groups based on morphological characters: a "northern group with spines on male metatarsi," a "northern group without spines on male metatarsi," a "southern group," and a "miscellaneous group." The species *Mesabolivar brasiliensis* (Moenkhaus 1898) and *Mesabolivar cyaneotaeniatus* (Keyserling 1891), both part of the "southern group," are distributed in southern and eastern Brazil and northern Argentina (Huber 2000, 2008).

Until now, only 15 pholcid species have been analyzed cytogenetically, and their diploid number varies from $2n♂$ = 15 to $2n♂$ = 32. The sex chromosome system (SCS) is commonly of the X type, but SCS of the X_1X_2 and X_1X_2Y types were also recorded. The meta/submetacentric morphology of the chromosomes predominates in the species karyotyped (see Oliveira et al. 2007). *Mesabolivar luteus* (Keyserling 1891), a representative of the "miscellaneous group," is the single species of *Mesabolivar* characterized chromosomally and its diploid complement was described as $2n♂$ = 15 = 14 + X (Araujo et al. 2005). Considering that *M. brasiliensis* and *M. cyaneotaeniatus* belong to the same *Mesabolivar* operational group, the aim of the present work was to determine and compare the mitotic and meiotic chromosomal characteristics of both species and to discuss the cytogenetic similarities with *M. luteus*, the karyotype of which was previously described by Araujo et al. (2005).

METHODS

Three embryos (one male and two females) and six adults (five males and one female) of *M. brasiliensis* collected at Estação Ecológica de Boracéia (22°11′S, 48°46′W), Salesópolis, state of São Paulo, Brazil and five males (two pre-adults and three adults) of *M. cyaneotaeniatus* collected at Universidade Estadual Paulista (UNESP), Rio Claro (22°41′S, 47°56′W), state of São Paulo, Brazil, were cytogenetically analyzed. The pre-adult and adult specimens were deposited in the collection of Arachnida in the Laboratório de Artrópodes, Instituto Butantan, São Paulo, state of São Paulo, Brazil (IBSP, A.D. Brescovit) (*M. brasiliensis* – IBSP 48221, 48223, 48229, 48234, 48235, 48241; *M. cyaneotaeniatus* – IBSP 75519, 75524, 75511, 75512, 75513).

Embryos and gonads were dissected in insect physiological solution (7.5g NaCl, 2.38g Na_2HPO_4, 2.72g KH_2PO_4 in 1L of distilled water), transferred to colchicine solution (0.05% for embryos and 0.16% for gonads, both in insect physiological solution) and left for 2 h; a volume of hypotonic solution (tap water) equal to that of the colchicine solution was added, and

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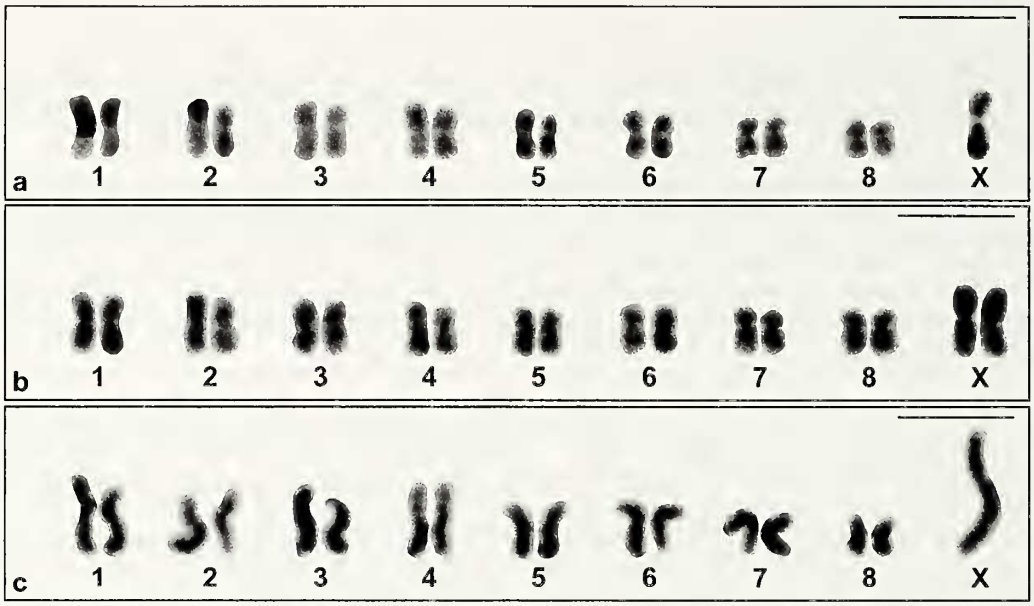

Figure 1.—Karyotypes of the *Mesabolivar* species standard stained with Giemsa: a, b. *Mesabolivar brasiliensis* with 2n♂ = 17 = 16 + X and 2n♀ = 18 = 16 + XX, respectively; c. *Mesabolivar cyaneotaeniatus* with 2n♂ = 17 = 16 + X. Note that in both species the X sex chromosome is the largest element of the complement. Scale = 10μm.

after 15 min, the material was placed in Carnoy I (3 methanol: 1 acetic acid) fixative solution for 30 min. The material was then transferred to a drop of 60% (embryos) or 45% (gonads) acetic acid on a microscope slide, and the material was macerated to form a cell suspension. The slide was dried on a metal heating plate at 35–40° C and most of them were stained with a 3% Giemsa solution for 13–15 min. Additionally, for detection of the AT- and GC- rich chromatin regions, some chromosomal preparations were stained with the fluorochromes 4′-6-diamidino–2–phenylindole (DAPI) and chromomycin A_3 (CMA_3), and counterstained with distamycin A (DA), according to the technique described by Schweizer (1980). The cells stained with Giemsa were photographed under a Zeiss microscope, using a Kodak Imagelink HQ Microfilm, and those stained with DAPI and CMA_3 fluorochromes were photographed under a Leica fluorescence microscope, using a Kodak T-Max 100 Film. The chromosomal morphology was determined following the nomenclature proposed by Levan et al. (1964).

RESULTS

The analysis of mitotic metaphases of male *M. brasiliensis* and *M. cyaneotaeniatus* revealed the karyotype with 2n = 17 = 16 + X (Fig. 1a, c). Only female specimens of *M. brasiliensis* were available for the study and showed a diploid complement of 2n = 18 = 16 + XX (Fig. 1b). There were no evident chromosomal size classes. Based on chromosome morphology, the elements of the karyotype were tentatively arranged in pairs in order of decreasing size. In both species, the X chromosome was always easily identified as the largest element of the karyotype (Fig. 1). In *M. brasiliensis*, the chromosomal morphology was established by means of embryo mitotic metaphase chromosomes, and all elements were classified as meta/submetacentric (Fig. 1a, b). In the case of *M. cyaneotaeniatus*, the male mitotic metaphases presented a less condensed condition, becoming impossible to determine the precise position of the centromere in the majority of the chromosomes (Fig. 1c). However, the analysis of metaphase II cells of *M. cyaneotaeniatus* revealed the meta/submetacentric morphology of all chromosomal elements (Fig. 2c, d). No evident secondary constrictions were found in mitotic metaphase chromosomes of either species.

The observation of diplotene eells of *M. brasiliensis* and *M. cyaneotaeniatus* revealed eight autosomal bivalents and one univalent, the X chromosome (8II+X), identified by its large size. In both species, each autosomal bivalent presented only one terminal or interstitial chiasma (Fig. 2a, b). The characteristics shown by diplotene cells of the two *Mesabolivar* species studied here confirmed the diploid number of 2n♂ = 17 and the SCS of the X/XX type in males and females, respectively. The metaphase II cells of *M. cyaneotaeniatus* exhibited nine and eight chromosomes (Fig. 2c, d). The cells with nine chromosomal elements included the X chromosome (n = 9 = 8 + X) that was always easily recognized by its large size and positive heteropycnosis (Fig. 2c); the cells with eight elements (n = 8) possessed only autosomes (Fig. 2d). These characteristics indicated the regular and reductional segregation of all chromosomes during anaphase I.

Due to the limited material available, only mitotic metaphases of *M. brasiliensis* were stained with base-specific fluorochromes. Mitotic chromosomes subjected to DAPI appeared to stain homogenously, with no differential bright region. On the other hand, the same chromosomes stained with CMA_3 showed bright fluorescence in the terminal region in the majority of the chromosomes, especially in the elements of pair 2. The X chromosomes were one of the exceptions, without differentially fluorescent regions (Fig. 3).

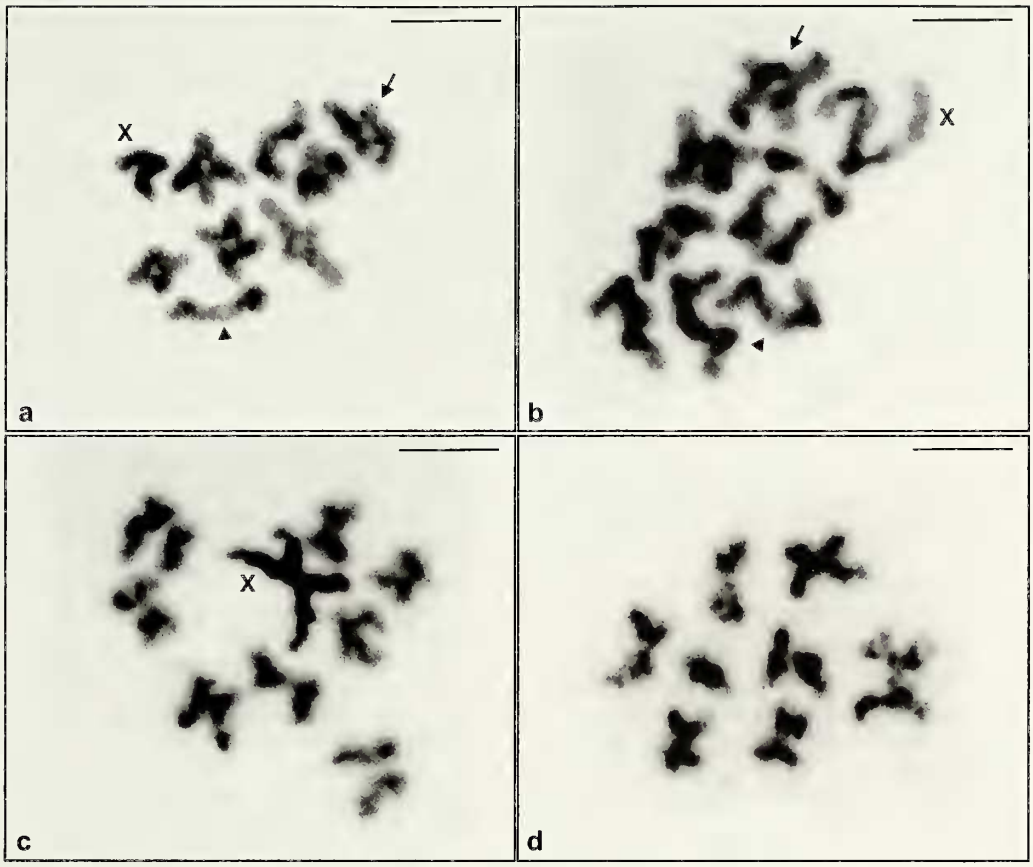

Figure 2.—Testicular meiocytes of the *Mesabolivar* species standard stained with Giemsa: a, b. Diplotenes of *Mesabolivar brasiliensis* and *Mesabolivar cyaneotaeniatus*, respectively, both with 8II + X, exhibiting one interstitial (arrow) or one terminal (arrowhead) chiasma per bivalent; c, d. Metaphases II of *M. cyaneotaeniatus* with n = 9 = 8 + X and n = 8 chromosomes, respectively, revealing that all chromosomes are biarmed. Scale = 10μm.

DISCUSSION

The general chromosomal characteristics of *M. brasiliensis* and *M. cyaneotaeniatus* agree with those found in other pholcid spiders that have been analyzed cytogenetically (see Oliveira et al. 2007). Concerning the diploid number, 2n♂ = 17 was first described for the pholcid *Micropholcus fauroti* (Simon 1887) (Araujo et al. 2005). However, the present addition of two more pholcids with the same number of chromosomes indicates that this diploid number could be as

Figure 3.—Mitotic metaphase of *Mesabolivar brasiliensis* with 2n♀ = 18 = 16 + XX stained with CMA_3 GC-specific fluorochrome, showing bright fluorescence in the terminal region of the majority of the autosomes. The arrows indicate prominent GC-rich DNA sequences in the pair 2 chromosomal elements. Scale = 10μm.

widespread as the $2n♂ = 15$, which has been recorded from five species of the family.

With regard to the SCS, the X type in males and XX in females found in both species here studied was also encountered in nine other pholcids (Bole-Gowda 1958; Cokendolpher 1989; Xiuzhen et al. 1997; Araujo et al. 2005; Král et al. 2006; Oliveira et al. 2007), and thus may be the most prevalent form in this family. In species of Pholcidae with X/XX system, the X chromosome seems to be the largest or nearly the largest element of the karyotype. This fact may indicate a single origin of this SCS in this group of spiders. An elegant hypothesis for the origin of the X system from the X_1X_2Y SCS in pholcids was already elaborated by Král et al. (2006).

The meta/submetacentric morphology of the chromosomes found in *M. brasiliensis* and *M. cyaneotaeniatus* agree with that described for the chromosomes of the majority of pholcid species karyotyped thus far, except for acrocentric X chromosomes of *Pholcus crypticolens* Bösenberg & Strand 1906 (Suzuki 1954) and autosomes of *Pholcus manueli* Gertsch 1937 (under *Pholcus affinis* Schenkel 1953) (Xiuzhen et al. 1997). Suzuki (1954) mentioned that he was able to obtain only a few slides of a quality sufficient to count metaphases and that it was difficult to determine even the chromosome number. It is possible that the morphology of the sex chromosomes has been interpreted incorrectly as acrocentric due to low chromosomal preparation quality. The problem of preparation quality in pholcid cytogenetical research has been highlighted by several authors (see Araujo et al. 2005). As an alternative to describe chromosomal morphology in species of this family, metaphase II chromosomes have been used (Araujo et al. 2005; Král et al. 2006; Oliveira et al. 2007; this work).

Our data from fluorochrome staining are the first from pholcid spiders. Terminal fluorescent regions in chromosomes, such those encountered in *M. brasiliensis*, were observed in species of the genus *Loxosceles* Heineken & Lowe 1832 (Sicariidae Keyserling 1880), other haplogyne species (Araujo pers. comm.).

Our comparison of the karyotype of *M. luteus* obtained by Araujo et al. (2005) with *M. brasiliensis* and *M. cyaneotaeniatus* revealed similar chromosomal morphology with biarmed elements (meta/submetacentrics) and the same SCS of the X/XX type. However, the diploid number differed among these three *Mesabolivar* species, that is, $2n♂ = 15$ in *M. luteus* and $2n♂ = 17$ in both species examined here. Huber (2000) placed *M. brasiliensis* and *M. cyaneotaeniatus* in the same operational group, the "southern group" that includes other species of the genus, and separated *M. luteus* and other *Mesabolivar* species in a "miscellaneous group." The latter group is composed of species that do not fit convincingly into any other genus and is certainly polyphyletic (Huber 2000). A molecular analysis of cytochrome oxidase I gene sequence in pholcids revealed a closer relationship between *M. brasiliensis* and *M. cyaneotaeniatus* than between *M. luteus* and any of these two species (Astrin et al. 2006). The chromosomal results corroborate the idea that *M. brasiliensis* and *M. cyaneotaeniatus* are more closely related to one another than either is to *M. luteus*. The revision by Huber (2000) indicated a probable close relationship between *Mesabolivar luteus* and *Mesabolivar levii* Huber 2000, both belonging to the "miscellaneous group."

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